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Effective Clinical Modulation of Knee Osteoarthritis Symptoms Through the Use of Combined Cellular Therapy Techniques

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COMBINED CELL THERAPY FOR THE TREATMENT OF KNEE OA

SUMMARY

The combination of autologous bone marrow aspirate concentrate (BMAC), fat graft, and platelet rich plasma (PRP), which we have defined as Combined Cellular Therapy (CCT), was utilized in the treatment of pain and functional deficits associated with osteoarthritis of the knee. This treatment has successfully modulated both pain and functional deficits associated with OA. 96 study subjects ages 38-90 with a total of 140 knees with a clinical diagnosis of mild (n=34), moderate (n=58), and severe (n=48) OA of the knee with no other complicating clinical diagnosis who completed follow ups at 6, 18, 52, and 104 week intervals were included in the study and received CCT injection(s) to one or both affected knees. Quantitative analysis of pain and function modalities were performed using the Visual Analog Scale (VAS) and the Knee Injury and Osteoarthritis Outcome (KOOS) Western Ontario and McMaster Universities Arthritis Index (WOMAC) respectively. Results of analysis showed that all study participants reported significant improvement ($p<0.001$) from baseline in VAS and WOMAC in mild to severe knee OA cases at 6 and 18 week follow ups, and also at yearly follow ups with mild and moderate OA. Severe OA had a return of some functional deficits as well as some return of pain but still significant improvement from baseline ($p<0.001$). Study results reveal that the use of CCT in mild to severe OA knee cases results in clinically significant improvement in both pain and functional scales, signifying cellular therapy as an orthopedic modality for the treatment of knee OA.

key words: cellular therapy, BMAC, PRP, knee, osteoarthritis, degenerative joint disease, regenerative medicine, fat graft

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INTRODUCTION

Osteoarthritis: Osteoarthritis (OA) is one of the most common chronic debilitating conditions seen in the orthopedic setting. Often, OA has a higher prevalence in the older and more obese populations particularly in joints that endure axial loading, more specifically the knees. Successful treatment of knee OA remains a challenge due to lack of blood supply and limited capacity of self repair in articular cartilage. Traditional treatment for our OA patients typically includes lifestyle modifications including transitioning to lower impact activities to reduce stress on joints, for those joints under axial loading. PT can help with range of motion and muscle strengthening. Unloader braces can be used instances where valgus or varus deformities are present (Birmingham et al., 2001; Brouwer, Jakma, Verhagen, Verhaar, & Bierma-Zeinstra, 2005). Assistive walking devices like canes or walkers are prescribed as well as heat and ice. Pharmacological intervention can be a part of traditional treatments for OA. Corticosteroid injections often provide temporary relief from pain and inflammation, but multiple injections over time have been shown to accelerate the progression of OA causing further damage (Ayhan, Kesmezacar, & Akgun, 2014). Oral anti-inflammatories can be prescribed alone or in combination with topical anti-inflammatories. When traditional conservative treatment options fail, surgical intervention is an option, with a total joint arthroplasty as the end point for treatment. Many patients, however, fall into a treatment gap in between traditional conservative treatment and major surgical intervention in which treatment options are limited. Little focus has been placed on regenerative options for orthopedics until now.

Mesenchymal Stem Cells*:* The use of mesenchymal stem cells (MSCs) derived from adipose tissue and bone marrow are currently under investigation in multiple research studies (Centeno et al., 2016; Emadedin et al., 2015; Jo et al., 2017; Jo et al., 2014; Shin, Yoon, Kim, & Lee, 2018; Wolfstadt, Cole, Ogilvie-Harris, Viswanathan, & Chahal, 2015). The therapeutic use of

MSCs and other reparative cells is traditionally related to both their anti-inflammatory activity and multilineage differentiation, including their chondrogenic potential. Bone marrow serves as a source for autologous MSCs that can differentiate into bone, cartilage, fat, and connective tissue cells, as well as release growth factors which decrease inflammation (Centeno et al., 2016; Kingery, Manjunath, Anil, & Strauss, 2019; Korbling & Estrov, 2003; Shin et al., 2018; Vega et al., 2015). It is believed that these adult cells retain a level of plasticity throughout their life that allows them to differentiate into various types of cells (Griffin, Iqbal, & Bayat, 2011). They have a role in tissue homeostasis by helping to repair and replace cells lost during injury or disease and seem to be highly malleable (Korbling & Estrov, 2003; Sekiya et al., 2012). Some researchers have found that the introduction of MSCs into the joint can help arthritic knees that may be deficient in stem cells and possibly result in regenerated cartilage (Jo et al., 2017; Minteer, Marra, & Rubin, 2013; Sekiya et al., 2012). Reparative adipose derived cells also have an immunoregulatory effect on the joint due to the paracrine factors that are secreted into the joint (Carelli et al., 2015; Minteer et al., 2013). Components of adipose tissue include the stromal vascular niche, extracellular matrix and numerous cell types including pericytes, pre-adipocytes, adipocytes and adipose derived stem cells as well as progenitor and hematopoietic cells (Carelli et al., 2015). A vital component in regenerative medicine is the pericyte, which exists in the stromal vascular niche of adipose tissue and plays an important role in cell signaling and healing (Ceserani et al., 2016). Recent clinical trials have shown the use of autologous stem cell therapies (either BMAC or adipose derived) in OA patients to be safe and effective in terms of improvements in cartilage regeneration, functional activity, and long term pain improvement (Pak, 2011; Pak, Lee, Park, Jeong, & Lee, 2016; Roseti, Desando, Cavallo, Petretta, & Grigolo, 2019; Shin et al., 2018). When compared to conservative treatment approaches, one study showed improvement in pain and evidence of meniscal regeneration with mesenchymal stem cell injections when compared to sodium hyaluronate injections

(Vangsness et al., 2014). Autologous mesenchymal stem cell injection were shown to be a promising minimally invasive therapy for osteonecrosis of the femoral head and knee osteoarthritis (Pak, 2011; Pak et al., 2016).

Platelet Rich Plasma: Platelet-rich plasma (PRP) is an increased concentration of autologous platelets in a small amount of plasma, containing growth factors and bioactive proteins that encourage a healing environment (Foster, Puskas, Mandelbaum, Gerhardt, & Rodeo, 2009). The processed product is at least 1 million platelets/μL in 5mL of plasma, though this number varies in each subject and is possibly influenced by age, gender or other comorbidities (Amable et al., 2013; Foster et al., 2009). PRP has a variety of growth factors that help to reduce inflammation in the joint while introducing chondroprotective cytokines to encourage chondrocyte proliferation, cell differentiation, and stimulating tissue regeneration at the site of degradation (Dai, Zhou, Zhang, & Zhang, 2017; Mascarenhas, Saltzman, Fortier, & Cole, 2015; Meheux, McCulloch, Lintner, Varner, & Harris, 2016). Growth factors are released once the PRP is introduced into the joint and can possibly be produced for 8-10 days after injection (Ayhan et al., 2014; Meheux et al., 2016). PRP has the potential to increase MSC proliferation and encourage the formation of cartilage repair tissue (Jin, Zhang, & Zhang, 2013). It is a vital component of the combined cellular therapy procedure that can be used on its own.

Combined Cellular Therapy: While these components in isolation have proven to be powerful means to modulate inflammatory response in osteoarthritis, we have shown that in combination, their interaction within the local microenvironment (niche) at the targeted injury site guides and further enhances the activation and proliferation toward site specific cell types and therefore enhanced reparative abilities (Darr & Daigle, 2016). The fat provides a living bioscaffold of cells

and stromal elements to which the BMAC adheres and modulates the inflammatory and reparative response (Tsubosaka et al., 2020). PRP further enhances these effects via cytokine secretion and activation of autocrine and paracrine pathways (Jin et al., 2013). In this prospective non-randomized clinical study of 96 patients with a clinical diagnosis of mild (n=34), moderate (n=58), and severe (n=48) OA of the knee with no other complicating clinical diagnoses, we quantified the clinical effectiveness of the combined use of autologous bone marrow aspirate concentrate (BMAC), fat graft, and platelet rich plasma (PRP), which we have defined as Combined Cellular Therapy (CCT) in the modulation of pain and functional deficits associated with knee OA. Each of these components provides different reparative properties and work in unison to achieve optimal results in pain reduction and functional enhancements.

MATERIALS AND METHODS

Participants: The current study includes 96 patients with a total of 140 knees with a clinical diagnosis of mild (n=34), moderate (n=58), and severe (n=48) OA of the knee with no other clinically complicating factors who have completed follow-ups at 6, 18, and 52 week intervals. All participants were submitted to an initial screening visit with a physical examination and knee radiography. Inclusion criteria were males and females over the age of 38 with a diagnosis of OA of the knee and confirmatory radiographs (Kellgren–Lawrence (KL) grade 2–4). Exclusion criteria were history of immunodeficiency, chronic use of oral corticosteroid or immunosuppressive therapies, history or presence of malignant disorders and/or use of chemotherapy within the last 5 years, except for cutaneous basal cell or squamous cell cancer resolved by excision, signs and symptoms of significant cardiac disease, diagnosis of transient ischemic attack within the last 6 months.

Platelet Rich Plasma: According to manufacturer's instructions for the Harvest SmartPRP Procedure Kit (, 54ml of whole blood were collected from all 96 patients with mild to severe knee OA with 6ml of ACD-A anticoagulant and double spun via centrifuge for a total of 14 minutes yielding approximately 7.0ml of PRP. The PRP sample was then placed in ADILIGHT for UV light activation for 10 minutes.

Lipoaspiration: According to the policies approved by the Institutional Review Boards for the Institute of Regenerative and Cellular Medicine ((A3-OA-901), adipose tissue was harvested from 96 patients with mild to severe knee OA. Written informed consent was obtained from all study participants. Under aseptic sterile conditions, stab incisions were made for cannula entry in the abdominal area and infiltrated with tumescent anesthesia fluid with 500ml of saline, 50ml of 2% lidocaine plus 1ml of (1:1000) epinephrine. Approximately 15 minutes following

infiltration, 50-70cc of adipose tissue was aspirated via cannula connected to a VacLock® (Merit Medical, South Jordan, UT, USA) syringe.

Lipoaspirate Processing: According to manufacturer's instructions for the Harvest AdiPrep Adipose Concentration System, the lipoaspirate was washed with sterile saline and spun via centrifuge for a total of 4 minutes yielding approximately 20cc of lipoaspirate. The concentrated lipoaspirate was then placed in the ADILIGHT for UV light activation for 10 minutes.

Bone Marrow Aspiration: According to the policies approved by the Institutional Review Boards for the Institute of Regenerative and Cellular Medicine ((A3-OA-901), bone marrow tissue was harvested from 96 patients with mild to severe knee OA. Written informed consent was obtained from all study participants. Under aseptic sterile conditions, a small stab incision was made over the iliac crest, a bone marrow trocar was introduced into the posterior superior iliac crest, and approximately 60cc of marrow was aspirated. The incision site was again cleaned, and then dressed with steri-strips and a small bandage.

Bone Marrow Aspirate Processing: The bone marrow aspirate (BMA) was placed in an injection filter bag, processed, and concentrated using the Harvest BMAC2 system. The BMA was spun via centrifuge for a total of 15 minutes yielding approximately 15cc of bone marrow aspirate concentrate (BMAC) and placed in the ADILIGHT for UV light activation for 10 minutes.

Intra-articular Injections: Under aseptic conditions, 11cc of 0.1% Lidocaine solution was injected into the knee(s) under ultrasound guidance. 7 cc of the activated lipoaspirate concentrate, 7 cc of the activated BMAC, and 7 cc of the activated PRP was then injected into

the knee joint(s) under ultrasound guidance. The site was dressed with simple bandages and a compressive cryo-therapy unit was applied.

Post-Operative and Post-Injection Care: Patients were discharged when stable with post procedure instructions. Prophylactic antibiotics were administered, and patients were monitored for fever and abnormal pain and swelling. Adjunct therapies of supplements and oral cytokines (GUNA® Biotherapeutics, Milan, Italy) were administered to the patient to enhance recovery and healing for a minimum of 6 weeks post injection. Patients were followed for a minimum of 1 year post Combined Cellular therapy and specifically at 6, 18, 52, and 104 weeks post therapy.

Patient Reported Outcome Measurements: A clinical and functional assessment was performed at each follow up interval. Patient reported outcomes of pain and function were measured using the Visual Analog Scale (VAS) and the Knee Injury and Osteoarthritis Outcome Score (KOOS) Western Ontario and McMaster Universities Arthritis Index (WOMAC) respectively (Bellamy, Buchanan, Goldsmith, Campbell, & Stitt, 1988; McConnell, Kolopack, & Davis, 2001). Deviations from baseline conditions were calculated and percentage improvements and/or decline were determined and quantitatively compared for each follow up time point. Data from patients with baseline pain and function scores less than 15/100 were excluded from the final analysis for this study, as scores of less than 15/100 were deemed not active disease and therefore treatment would be considered prophylactic. Based on this screening threshold, data from 116 knees were included in the final analysis with (n=23) Kellgren Lawrence (KL-II), (n=49) KL-III, and (n=44) KL-IV.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism 8 (GraphPad Prism, LLC, San Diego, CA, USA). The level of significance for all hypothesis tests (*p*) was set at 0.05. Continuous variables were presented as mean and standard error. Comparisons of knee VAS and KOOS WOMAC scores were independently made with the Kruskall–Wallis test for each data set. Once the Kruskal-Wallis test showed statistical significance among all normalized timepoints, post-hoc analysis was performed using the Wilcoxon signed-rank test to delineate the improvement of measurements between each endpoint with 95% confidence intervals (CI). Patient reported outcome measurements of KOOS WOMAC scores measured during each follow-up endpoint for among OA severity groups were also quantitatively compared utilizing the Mann-Whitney test to reveal differences among KL severity.

Ethical Approval: This study was reviewed and approved for human studies by the International Review Board for Cellular Medicine. All patients signed a detailed informed consent, which was also reviewed and approved by the IRB. There was no funding provided to the investigator, and no patient compensation for participation.

RESULTS

Patient Reported Outcomes: At the 6 week follow up, all study participants reported significant improvement from baseline with 56.34 ± 7.42% (mean ± SE) improvement in VAS and 35.88 ± 11.95% improvement in WOMAC in mild OA cases, 37.01 ± 6.51% improvement in VAS and 49.23 ± 4.64% improvement in WOMAC in moderate OA cases, and 51.06 ± 6.85% improvement in VAS and 42.60 ± 5.88% improvement in WOMAC in severe OA cases . These early results continued to progress in the mild OA group through 18 weeks with 62.47 ± 5.38% improvement in VAS and 47.47 ± 8.65% in WOMAC and at the yearly follow-up 71.42 ± 5.56% improvement in VAS and 65.54 ± 7.98% improvement in WOMAC. The patients with moderate OA demonstrated significant functional improvements at the 18 week follow-up with 56.68 ± 5.60% improvement in VAS and 59.46 ± 4.71% improvement in WOMAC that were maintained at the 1 yr follow-up with 59.53 ± 4.48% improvement in VAS and 48.38 ± 8.13% improvement in WOMAC. The patients with severe OA showed significant improvement through 18 weeks with 40.86 ± 9.23% improvement in VAS and 48.38 ± 6.42% in WOMAC and at the yearly follow-up with 27.46 ± 9.54% improvement in VAS and 24.03 ± 1.00% improvement in WOMAC as illustrated in **Figure 1**. All 116 patients completed the 1 year follow up and 88% of those patients showed improvement in pain and function. 47 of the 116 patients completed the 2 year follow up and 95% of those patients showed improvement in pain and function. Statistical comparisons of knee VAS and KOOS WOMAC scores were independently made with the Kruskall–Wallis test for each data set, revealing statistically significant changes from baseline values among all data sets for mild, moderate and severe OA cases with $p<0.001$ in all cases. Post-hoc analysis was then performed using the Wilcoxon signed-rank test to delineate the improvement of measurements between each endpoint with 95% confidence intervals (CI). Post hoc analysis for Mild, moderate, and severe OA cases revealed statistical improvement in VAS and WOMAC scores through 18 weeks with a maintenance of those results up to a year

and improvement thereafter, at 2 years post CCT therapy. While the trends showed continual improvement over time for mild OA cases, there appeared to be a plateau of maintained improvement from 18 weeks up to 2 years for moderate OA. Interestingly, the trends revealed improvement up to 18 weeks for severe OA with a maintenance of those results or slight worsening at a year post, followed by a seemingly delayed onset of symptom improvement between 1-2 years post CCT therapy. To account for any data skewing caused by the loss of retention of patients at the 2 year follow up, we decided to examine these numbers more closely with a more stringent paired analysis of these data points.

Paired analysis of year 1 and year 2 data among all OA groups revealed no statistically significant difference, as shown in **Figure 2**. Trendlines suggest improvement at year 2 for the severe OA group, and likely would reflect this with larger retention of study subjects. Current and futures studies have been modified to enhance retention and increase compliance with collection of patient reported outcome measurements. Yearly outcome correlates among groups (mild, moderate, and severe OA) were made of the patient reported outcome measurements of VAS and KOOS WOMAC scores for each time point measured and were quantitatively compared utilizing the Mann-Whitney test and showed significant enhanced improvement in 1 year post CCT VAS scores in Mild OA vs Severe OA with $p<0.001$ and enhanced improvement in Moderate OA when compared to severe OA in year Vas scores with $p<0.01$. No significant difference was found between mild and moderate OA yearly VAS results as shown in **Figure 3**. Statistically signifcant improvement in 2 year post CCT VAS scores in Mild OA vs Moderate OA with $p<0.01$. No other significant difference was found between mild and severe and severe and moderate 2 year VAS results. Results also showed significant improvement in 1 year post CCT WOMAC scores in Mild OA vs Severe OA and vs Moderate OA with $p<0.01$ and improvement in moderate OA when compared to severe OA in year VAS scores with $p<0.01$. Analysis of 2 year post CCT WOMAC results of showed significant

improvement in 2 year post CCT WOMAC scores in Mild OA vs Moderate OA and mild vs severe with $p<0.01$. No significant difference was found between moderate and severe 2 year WOMAC results.

DISCUSSION

The results of this study affirmed our hypothesis that combined cellular therapy can greatly reduce pain and increase function in patients diagnosed with OA of the knee. Combined cellular therapy can serve as an alternative method of conservative treatment for patients who are unwilling or unable to have surgery. The combination of bone marrow aspirate concentrate, activated platelet-rich plasma, and the fat graft shows potential to provide patients with pain relief and slow down degradation caused by OA. This is especially true for patients who use cell therapy as an early intervention. Degradation of cartilage and bone caused by OA may be slowed and possibly halted in some cases, particularly for those who use the therapy as an early intervention. Our results showed that patients with lower grades of OA saw greater and longer lasting benefits from combined cell therapy than those with more advanced OA.

Though there continues to be more evidence published supporting the success of cellular therapy in the treatment of OA (Centeno et al., 2016; Chahla et al., 2016; Diekman & Guilak, 2013; Emadedin et al., 2015; Jo et al., 2014; Kingery et al., 2019), further research on larger populations with longer follow-up periods is needed. Participants being lost to long term follow-up were one of the major limitations of this study, but these results support the use of CCT as an alternative method of conservative treatment for patients who are unwilling or medically unable to undergo surgery or want surgery as a last resort. If administered early, these treatments may lead to longer lasting benefits and less likely developing progressive OA, signifying its potential use as a prophylactic treatment.

CONFLICT OF INTEREST

Neither author has any proprietary interests in the materials described herein and no conflicts of interest to disclose.

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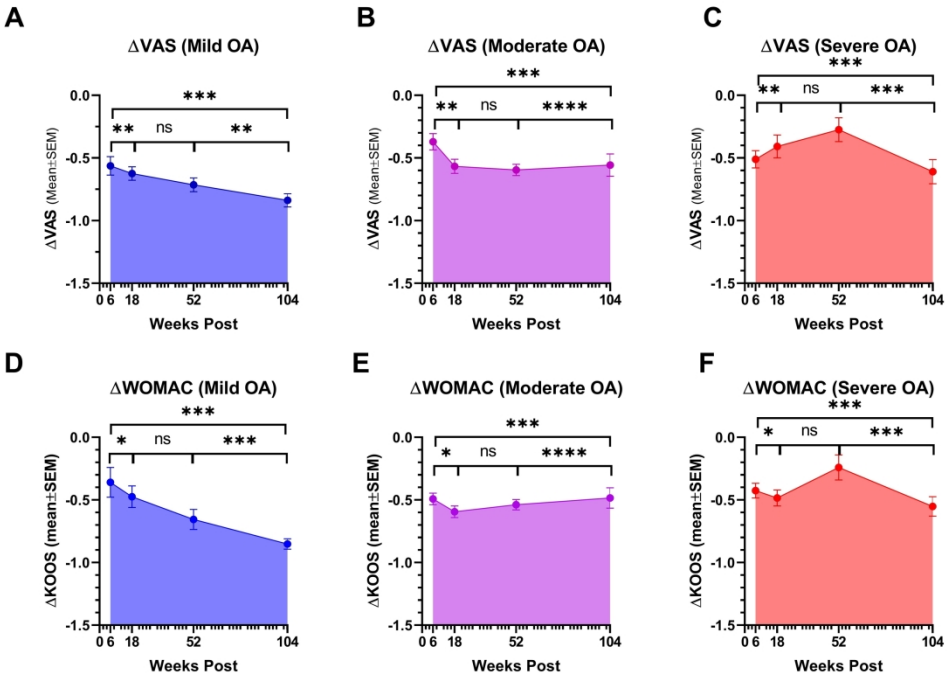
FIGURE LEGENDS

Figure 1: Patient reported outcome measurements of VAS and KOOS WOMAC scores for mild knee OA in blue (**A** and **D**), moderate knee OA in purple (**B** and **E**) and severe knee OA in red (**C** and **F**) measured during each follow-up endpoint. Quantitative comparative analysis was performed utilizing the Kruskall–Wallis test, revealing statistically significant changes from baseline value among all data sets for mild, moderate and severe OA cases with $p<0.001$*** in all cases as indicated on the top line in each graph. Post-hoc analysis was then performed using the Wilcoxon signed-rank test to delineate the improvement of measurements between each endpoint with 95% confidence intervals (CI).

Figure 2: Plots of parallel bar graphs of the stringent paired analysis showing 1 year and 2 year results of mean percentage improvement of patient reported outcome measurements of VAS (**A**) and KOOS WOMAC (**B**) for mild knee OA (blue), moderate knee OA (purple), and severe knee OA (red).

Figure 3: Normalized deviations from baseline (mean±SEM) of 1 year and 2 year VAS scores (**A** and **C**) and WOMAC scores (**B** and **D**) for mild knee OA (blue), moderate knee OA (purple), and severe knee OA (red). Approximate mean percentage improvements are overlaid on top of plots for clarity. Statistical significance as results of Mann-Whitney comparisons are overlaid on plots.



Patient reported outcome measurements of VAS and KOOS WOMAC scores for mild knee OA in blue (A and D), moderate knee OA in purple (B and E) and severe knee OA in red (C and F) measured during each follow-up endpoint. Quantitative comparative analysis was performed utilizing the Kruskall–Wallis test, revealing statistically significant changes from baseline value among all data sets for mild, moderate and severe OA cases with $p<0.001$*** in all cases as indicated on the top line in each graph. Post-hoc analysis was then performed using the Wilcoxon signed-rank test to delineate the improvement of measurements between each endpoint with 95% confidence intervals (CI).

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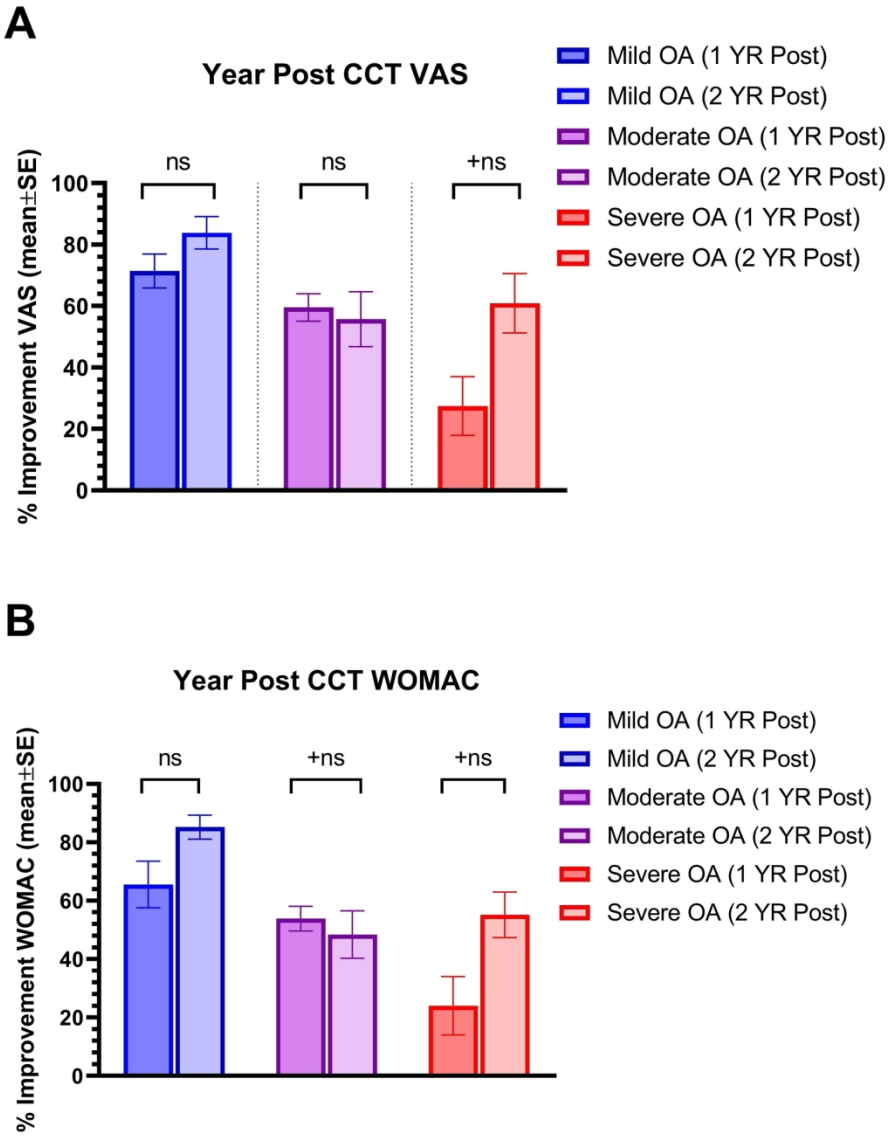
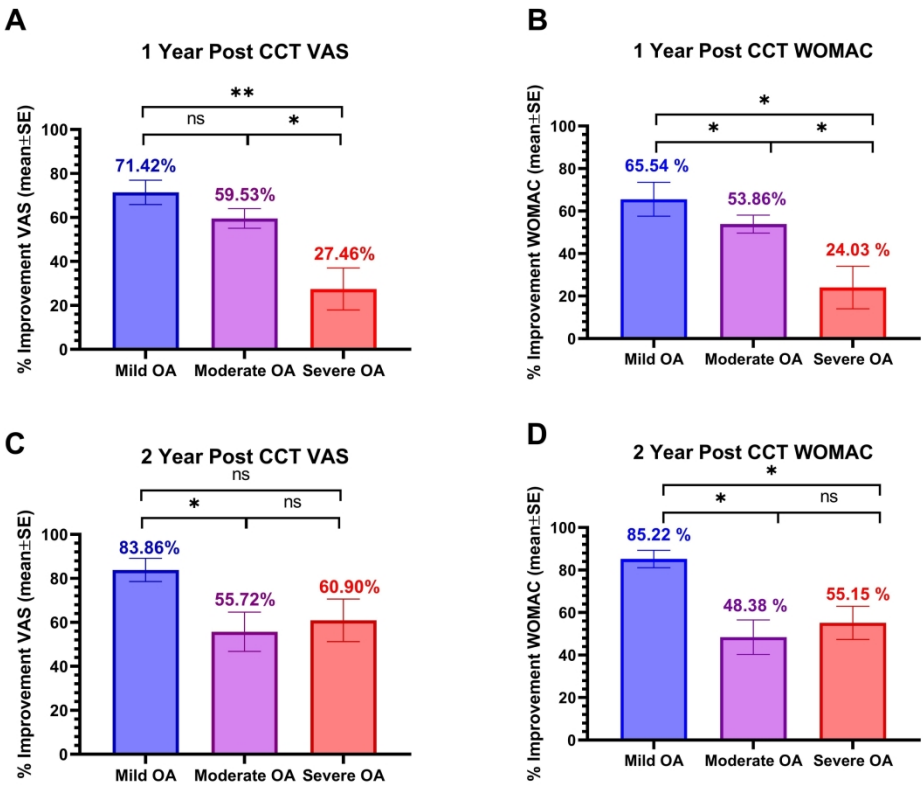


Plots of parallel bar graphs of the stringent paired analysis showing 1 year and 2 year results of mean percentage improvement of patient reported outcome measurements of VAS (A) and KOOS WOMAC (B) for mild knee OA (blue), moderate knee OA (purple), and severe knee OA (red).

152x189mm (300 x 300 DPI)



Normalized deviations from baseline (mean±SEM) of 1 year and 2 year VAS scores (A and C) and WOMAC scores (B and D) for mild knee OA (blue), moderate knee OA (purple), and severe knee OA (red). Approximate mean percentage improvements are overlaid on top of plots for clarity. Statistical significance as results of Mann-Whitney comparisons are overlaid on plots.

236x195mm (300 x 300 DPI)